Repellent effect of plant essential oils against *Aedes albopictus*

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ABSTRACT: Six essential oils: asteraceae oil, rutaceae oil, mentha piperta oil, carvacryl oil, citronella oil, and eucalyptus oil were tested for evaluation of their repellent effects against *Aedes albopictus* mosquitoes under laboratory conditions. Only citronella oil and eucalyptus oil were tested with human beings. There was considerable protection for mice. Carvacryl oil (7%) provided 100% protection for mice after 7 h. Eucalyptus oil (15%) gave protection to humans for least 3 h; the protection time was prolonged to 5 h after adding 5% vanillin. The mixture could be developed into a practical product after the field evaluation. ***Journal of Vector Ecology* 30 (2): 231-234. 2005.**

Keyword Index: Repellents, essential oils, *Aedes albopictus*.

INTRODUCTION

Several mosquito species can spread pathogens of diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, and yellow fever (Su and Ye 1996). Currently, major approaches to these mosquito-borne diseases are mosquito control and personal protection from mosquito bites. Repellents are a practical and economical means for preventing the transmission of these diseases to humans. Repellents, such as the "gold standard" *N,N*-diethyl-3-methylbenzamide (DEET), has shown significant repellency against mosquitoes and other biting arthropods (Yap 1986, Coleman et al. 1993, Walker et al. 1996). However, toxicity reactions for humans after applications of DEET can be severe (Zadikoff 1979, Robbins and Cherniack 1986, Edwards and Johnson 1987, Qiu et al. 1998). To avoid these adverse effects, many laboratories have tried to replace DEET with repellents that are derived from plant extracts.

Various plant extracts, such as neem (from *Azadirachta indica* A. Juss), basil oil (*Ocimum basilicum* L., *O. basilicum* L. fa. *citrtum* Bach, *O.* gratissimum L., *O. americanum* L., *O. tenuiflorum* L.), citronella grass (*Cymbopogon nardus* Rendle), galingale (*Alpinia galanga* L.), clove (*Syzygium aromaticum* L.), and thyme (*Thymus vulgaris* L.), have been recorded as mosquito repellents (Sukumar et al. 1991, Sharma et al. 1993, Chokechaijaroenporn et al. 1994, Suwonkerd and Tantrarongroj 1994, Boonyabancha et al. 1997, Barnard 1999). Several natural repellents have demonstrated good efficacy against some mosquito species, but they were evaluated only with mice under laboratory conditions. It would be better to conduct the research on humans, although this is difficult.

In this study the repellency of several essential oils against *Ae. albopictus* was tested on mice and humans, searching for potential essential oils with promising protective effects on humans.

MATERIALS AND METHODS

Essential oils

Six essential oils were chosen for this study: asteraceae oil was extracted from *Ajania tenuifolia* collected from the Haibei Alpine Meadow ecosystem, Qinhai Province, China; Rutaceae oil was extracted from leaves of *Citrus sinensis* collected from Deqing County, Guangdong Province, China. These two were both extracted by steam distillation. Mentha piperta oil, carvacryl oil, citronella oil, eucalyptus oil and vanillin were the products of Shanghai Charoma Perfumery and Bio-Chem Co., Ltd. China, and the oils' source plants were *Mentha piperita, Mentha spicata*, *Cymbopogon citrates,* and *Eucalyptus globulus*, respectively. All testing solutions were dissolved in ethanol.

Test mosquitoes

The mosquitoes were a laboratory colony of *Ae. albopictus* provided by Jiangsu Institute of Parasitic Diseases Control, China. The mosquitoes were maintained at 26°C ± 1°C, 65% ± 5% (RH), and 12 h:12 h (light:dark) photoperiod. Nulliparous females of 4-5 days-old were used, and all testing was carried out in the rearing room. The same cage of mosquitoes was used to test the same oil solutions. The repeated tests were carried out with different mosquitoes. Toxicity on mosquitoes was not observed during the testing procedure.

Repellency test on Kunming mice

Kunming mice were fixed supinely on the board with their abdomens cleaned and depilated. An exposed hairless area of 2 × 2 cm was marked and the mouse was put into a mosquito cage (40×30×30 cm, containing 300 female mosquitoes) for 2 min. If more than 20 mosquitoes bit the mouse during a test, the mosquitoes and mouse were then used in the repellency tests. Five essential oils were tested at a concentration of 7%; two of them were also tested at 1% and 15%.

The testing solution (5 µl/cm^2) was painted on the exposed part of the mouse abdomen. After 1 h, the mouse was put in the mosquito cage for 2 min, removed, and then put again into cages every hour for 7 h. The number of blood-fed mosquitoes was observed. The blank control consisted of 100% ethanol. Each treatment was repeated three times.

The percentage of protection for the mice was calculated by the formula below (Frances et al. 2001):

Percentage of protection (100%) = [(Control - Treated) / Control] ×100

Controls consisted of mosquitoes that fed on mice treated with a control solution, compared to the number bitten treated by the testing solution. The data analysis used was a repeated measures analysis of variance (ANOVA) and completed by Statistical Analysis Systems (Version 8.2).

Repellency test on humans

Two essential oils were tested at concentrations of 15% and 30%. The mixture of 5% vanillin and testing solution was also tested. An area of 4 × 4 cm on volunteers' hands was marked and painted with a testing solution (2 µl/cm^2), while the other hand was treated with the mixture. The hand was covered by plastic film except for the marked area. After 1 h, each volunteer put his or her hand into a mosquito cage (40×30×30 cm, containing 300 nulliparous females) for 2 min, then removed it and again placed it into the cage for another 2 min every hour for 7 h. The test continued until one mosquito bit a volunteer. The control was 5% vanillin and 4% DEET ("Wen Bu Ding" solution, containing 4% DEET). Proboscis amputated mosquitoes that cannot feed were used on human subjects (Shirai et al. 2000). If there was no single mosquito biting during the time after application of the test solution, it was recorded as protecting (+), otherwise, as not protecting (-) .

RESULTS

Protection on Kunming mice

The protection results on Kunming mice treated by 7% essential oils against *Ae. albopictus* is shown in Table 1. There was no repellency with the control (100% ethanol). The protection time of carvacryl oil was the longest and the percentage of protection remained 100% after an exposure of 7 h. The protection percentage of five essential oils was more than 90% after 7 h treatments. There were significant differences among the five essential oils by repeated measures analysis of variance (F=9.78, 4 df, P = 0.0009), and there was also significant variance among the different time sections (F = 6.31, 7 df, P=0.0009). The repellency of the five essential oils against *Ae. albopictus* was high during the first 3 h, and there was no significant difference among those exposed for 4 h to 8 h. There was no significant correlation between the time section and essential oils (P = 0.1742).

There was no significant difference in repellency between asteraceae oil solutions (F = 0.22, 2 df, P = 0.8122) and rutaceae oil solutions (F=2.31, 2 df, P=0.1806). Their protection rate all exceeded 87%. A repeated measures analysis of variance revealed that there were significant differences among the three concentrations (1%, 7%, and 15%) of asteraceae oil (F=3.79, 7 df, P=0.0032) and rutaceae oil (F=11.71, 7 df, P<0.0001) with respect to time. The repellency of asteraceae oil was the highest (F=27.27, P=0.0020; F=2.31, P=0006) at an exposure of 3 h and wore off after 8 h (F=10.07, P=0.0193). Rutaceae oil was most potent (F=53.89, P=0.0003; F=25.53, P=0.0023; F=48.73, P=0.0004; F=23.90, P=0.0027) at an exposure of 4 h and wore off markedly after 7 h (F = 43.41, P = 0.0006). There was no correlation between concentration and exposure time (F=0.49, 14 df, P=0.9205) in asteraceae oil, but this correlation was significant (F=3.51, 14 df, P=0.003814) in rutaceae oil.

Protection on humans

The results of protection on human beings against *Ae. albopictus* by the essential oils is shown in Table 2. The range of protecting periods were 1 h, 3 h, and 1 h with 30% citronella oil, 15% and 30% eucalyptus oil solution, respectively. There was no repellency activity with 15% citronella oil solution. However, the repellency activity increased significantly when 5% vanillin was added to the testing solution. As a result, the range of protecting periods was extended to 3 h and 2 h with 15% and 30% citronella oil solution, respectively, and to 5 h and 4 h with 15% and 30% eucalyptus oil solution, respectively. The control solutions were 4% DEET and 5% vanillin solution. The 4% DEET solution provided protection for at least 6 h, and 5% vanillin solution for only 1 h.

DISCUSSION

Five oil solutions had considerable protection for mice in this study. The repellency was related to the type of essential oil and exposure time, but there was no significant correlation between them, which showed that the pattern of response in mosquitoes to repellent materials over time was not different among the essential oils. The results using the three concentrations of asteraceae oil and rutaceae oil showed that the repellency was not related to concentrations. It is suggested that carvacry oil would be suitable as a candidate for mosquito repellents. However, there was a difference between human and mouse skin after being treated with the same oil solution. There was not any protection to humans with the above oil solutions, except citronella oil, even if the concentration was above 30%.

Citronella oil was chosen to test on volunteers after screening on mice. Eucalyptus oil was tested previously (Trigg 1996, Moore et al. 2002, Hadis et al. 2003, Schreck and Leonhardt 1991). Eucalyptus-based repellent containing 30% *p*-menthane-diol had a protection rate of 96.89% for 4 h against *Anopheles darlingi,* which was similar to those currently in application (Moore et al. 2002). In the present study, the protection rate of 15% eucalyptus oil with 5% vanillin was similar to that of 4% DEET, even though their effects were different in many respects. Citronella oil cannot reliably protect humans, as reported by Wasuwat et al. (1990),

Table 1. Repellency effects of five essential oils on Kunming mice against *Aedes albopictus*.

Time of exposure	Percentage of protection (%)											
	control *	carvacryl oil (7%)	mentha piperta oil (7%)	citronella oil (7%)	asteraceae oil				rutaceae oil			
					control*	1%	7%	15%	control *	1%	7%	15%
1 h	23	100.00	100.00	100.00	20	97.27	99.67	95.00	20	99.66	99.67	100.00
2 h	21	100.00	100.00	100.00	26	98.31	99.67	95.56	20	99.67	100.00	99.67
3 h	23	100.00	100.00	100.00	20	98.99	99.67	97.09	22	99.67	98.66	100.00
4 h	27	100.00	100.00	99.67	21	94.80	94.55	96.31	21	100.00	98.99	98.99
5 h	27	100.00	99.00	99.67	20	93.74	94.86	95.32	18	97.63	96.29	99.33
6 h	25	100.00	99.33	99.67	26	95.83	96.26	96.23	20	98.26	98.00	97.72
7 h	27	100.00	99.00	99.67	25	93.82	95.80	92.85	20	96.58	96.40	87.29
8 h	20	98.33	97.67	99.67	18	90.35	91.81	93.28	15	98.31	95.81	95.54

*Number of blood-fed mosquitoes.

Table 2. Repellency effects of two essential oils and control treatment against *Aedes albopictus* on humans.

Time of exposure	citronella oil				eucalyptus oil				vanillin (5%)	DEET (4%)
	15%	15%+ 5% vanillin	30%	30% + 5% vanillin	15%	15% + 5% vanillin	30%	30% + 5% vanillin		
1 h	-	+	+	+	+	+	+	+	+	+
2 h	-	+	-	+	+	+	-	+	-	+
3 h	-	+	-	-	+	+	-	+	-	+
4 h	-	-	-	-	-	+	-	+	-	+
5 h	-	-	-	-	-	+	-	-	-	+
6 h	-	-	-	-	-	-	-	-	-	+
7 h	-	-	-	-	-	-	-	-	-	-

+: protecting, -: non protecting.

and Suwonkerd and Tantraongroj (1994). It is important to note that citronella oil in the above studies was obtained from different sources. In fact, repellency of a cream containing 14% citronella oil could keep *Ae. aegypti* away for 2 h (Wasuwat et al. 1990), while 10% citronella oil could keep *An. minimus* away for less than 2 h under laboratory conditions (Suwonkerd and Tantraongroj 1994). Unfortunately, this laboratory study was conducted during the day whereas *An. minimus* feeds at night. We also observed that the essential oil solution had repellency against *Cx. pipiens quinquefasciatus* and *An. stephensi* but had no repellency against *Ae. albopictus* (unpublished data).

The protection time of the two oils can be significantly increased by the incorporation of 5% vanillin. This agrees with data that vanillin can prolong protection against *Ae. aegypti* in most cases (Khan et al. 1975). Based on this study, it is suggested that the mixture of 15% eucalyptus oil with 5% vanillin can be developed into a practical product after field evaluation.

Essential oils contain complex constituents. Their components are decided by geographical distribution, harvesting time, and growing conditions like soil, water, and nutritional conditions, and their repellent mechanism is still not very clear. The major components must be analyzed if the repellent mechanism were to be studied.

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